HYDROCHEMICAL FACTORS LIMITING THE DISTRIBUTION OF *BULINUS TRUNCATUS* (PULMONATA: PLANORBIDAE)

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ABSTRACT

While extremely low calcium to magnesium ratios sometimes exclude the presence of *Biomphalaria* spp., at 53 sampling stations in the south Tunisian schistosomiasis distribution area, these ratios are in a range that evidently does not exceed values tolerated by *Bulinus truncatus* (Audouin). Only very high concentrations [Ca^{++} >425 ppm; Mg^{++} >135 ppm; Cl^->600 ppm; electrical conductivity (18ºC) >2440 μmho] were avoided. The Ca/Mg ratios in Tunisia were between 0.63 and 3.4. Laboratory snails showed a significant decrease in egg laying rates in Ca/Mg from 0.5/1 to 0.2/1, and to nil at 0.1/1. Ratios were varied by addition of $MgCl_2$ to a synthetic medium containing 100 ppm calcium and the other main ions at world mean ratios. Long term maintenance (over 1/2 year) of snails at ratios <0.75/1 resulted in a cessation of reproduction. When the Ca/Mg ratio (meq/meq) was kept constant at 3.65, which is the world mean ratio, and the absolute chloride concentration was raised by addition of appropriate amounts of the chlorides of calcium and magnesium, the egg laying rates remained high, up to 334 ppm, but significantly decreased in chloride concentrations of 866 and 1752. Natural water from Gabès, where *B. truncatus* did not occur, prevented egg laying and was lethal to experimental snails. It became suitable for egg laying by dilution with deionized water. It is concluded that high absolute concentrations of electrolytes, particularly chlorides, limit the distribution of *B. truncatus* in Tunisia and probably in other arid countries. A chloride concentration of about 600 ppm appears to form the upper threshold, as judged from both field and laboratory findings. *B. truncatus* appears to tolerate dissolved calcium and magnesium at relatively high levels, while *Biomphalaria pfeifferi* (Krauss), and probably other *Biomphalaria* species, prefer soft to medium hard water. In Tunisia, several freshwater prosobranch snails are able to live at yet higher electrolyte contents (electrical conductivity up to 10500 μmho; Ca^{++} up to 626 ppm; Mg^{++} up to 220 ppm; Cl^- up to 3900 ppm). Prosobranchs have probably maintained physiological capacities similar to their marine relatives, whereas freshwater pulmonates have attained a greater physiological distance from their marine ancestors.

The calcium to magnesium ratio in water is, as a rule, much greater than 1/1 in temperate climates. When it is extremely low, e.g. in dolomite areas, it can exclude the presence of schistosome host snails. Harrison *et al.* (1966) found this to be the case for *Biomphalaria pfeifferi* (Krause) in Zimbabwe. The adverse effect was not caused by high absolute magnesium concentration. Addition of calcium chloride brought the ratio to balance (weight ratio, corresponding to an equivalent rate of 0.6/1 and rendered the water suitable for the snails as expressed by significant increases in egg laying rates.

The existence of magnesite mining in Tunisia led us to examine the variation of Ca/Mg and its potential influence on the presence or absence of *Bulinus truncatus* (Audouin), a schistosome snail host. Other possible factors limiting distribution were also examined.

MATERIALS AND METHODS

Bulinus truncatus were reared from stocks collected by D. Haas in Gafsa (34º28′N, 8º43′E), central Tunisia, March 1970, and by J. Rutschke in Arak Bordj (25º20′N, 3º46′E), Algerian Sahara, February 1979. Laboratory studies were performed in 1970/71 on Tunisian snails and in 1981/82 on the Algerian stock.

Culture media for the examination of varied Ca/Mg ratios were obtained by adding appropriate quantities of magnesium salts to synthetic standard freshwater (SFW 100, containing 100 ppm calcium; for other details of composition of this medium, which represents world mean ratios of main ions, see Meier-Brook, 1978). Since magnesium carbonate is unstable and unobtainable, variation of magnesium concentration was achieved with $MgCl_2$ or $MgSO_4$. Snails were reared and used in the studies in SFW 100 at 25 ± 1°C and with a 12/12 hr light-dark regime. Fresh lettuce was fed daily *ad libitum*. Media were changed once a week unless otherwise stated. Media were aerated through hypodermic needles connected to an aquarium pump.

Snails were collected and water sampled in spring 1970 and 1971. Hydrobiid taxonomy follows that compiled by Boeters (1976). The 64 sampling stations were located in five areas in southern Tunisia, mainly around Chott Djerid, which itself seems to be free of mollusks (see Haas, 1973). Temperature, pH (Metrohm E 444), alkalinity and total hardness (Titriplex A Merck) were determined immediately; electrical conductivity at 18°C (wtw. L.F. 54), calcium, magnesium (both Titriplex), and chloride ($AgNO_3$ titration, indicator K_2CrO_4) in the laboratory in Tübingen. Carbonate hardness was calculated from alkalinity values.

RESULTS

Although the egg laying rate (Fig. 1) in the Sahara strain (Algeria) was very low, there is a tendency to reduce egg laying in Ca/Mg ratios below 0.75 down to zero at 0.1. When the Ca/Mg ratios were varied by adding the sulphate of magnesium, egg laying was further reduced to values as low as 0.0007 in 0.1/1 to 0.08 in 3.65/1. The Tunisian strain, on the other hand, had a considerably higher egg laying rate (Fig. 2). A non-significant increase occurred when $MgCl_2$ was added up to a ratio 1/1. A ratio of 0.5/1 resulted in egg laying rates almost equal to that in standard freshwater (SFW, ratio 3.65/1). A significant (t-test: $p<0.05$) decrease in rate occurred at the ratio of 0.2/1, and in 0.1/1 (the replicate only) no eggs were laid.

Long-term maintenance over 17 weeks, with a final reading after 27 weeks (Fig. 3) eventually yielded, despite heavy fluctuations, a decrease of survivorship with Ca/Mg ratios (varied by $MgSO_4$) below 1/1 and an extinction, after the ninth week, at 0.1/1. At the end of the experiments reproduction had ceased at ratios of 0.5/1 and less.

From sampling stations in Tunisia where the water had

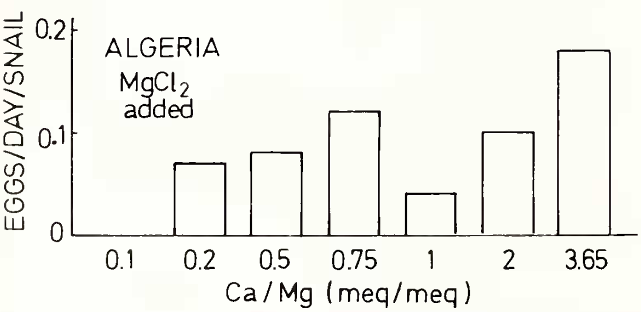

Fig. 1. Egg laying rates in Algerian *Bulinus truncatus* in artificial media with varied Ca/Mg ratios. Four beakers with 125 ml medium and 6 snails of 6 to 7 mm height each. Ratios varied by addition of $MgCl_2 \cdot 6 H_2O$ to SFW 100. Total chlorides are (from 0.1 to 3.65): 1752; 866; 344; 216; 157; 68; 28 ppm. Mean values of counts over four weeks.

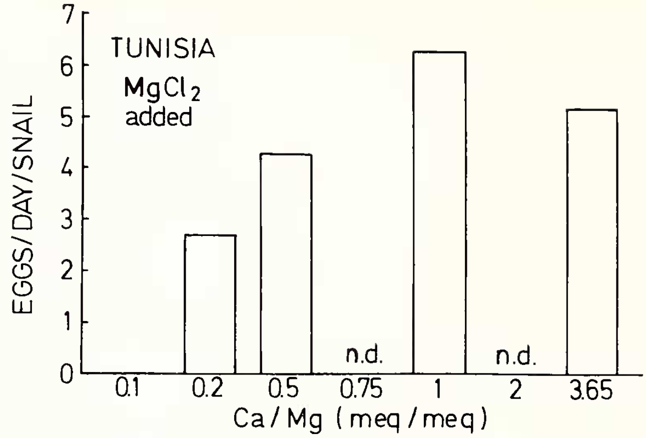

Fig. 2. Egg laying rates in Tunisian *Bulinus truncatus* in artificial media. One replicate. Ratios varied by addition of $MgCl_2 \cdot 6 H_2O$ to SFW 100 (0.1/1 only in the replicate). Total chlorides see figure 1. Mean values of counts over three or (replicate) four weeks, 4 x 4 snails, 8 to 9 mm high, in 100 ml medium each.

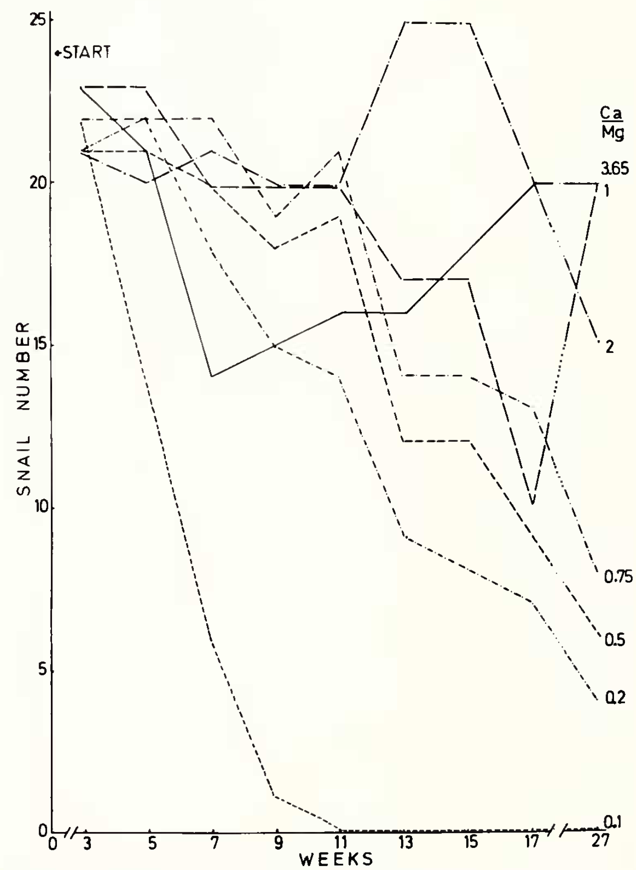

Fig. 3. Population development in Algerian *Bulinus truncatus* in artificial media with graded Ca/Mg ratios. Ratios varied by addition of $MgSO_4 \cdot 7 H_2O$. Every two weeks all snails of > 2 mm maximum diameter were counted. Sums of counts in 4 beakers containing 125 ml of medium and snails of 2 to 3 mm initial diameter. Total sulphates are (from 0.1 to 3.65): 2396; 1195; 475; 315; 235; 115; 60.5 ppm.

Table 1. Occurrence of Tunisian gastropods according to total electrolytes expressed as electrical conductivity (μmho at 18°C).

Species	Range	1220 - 2500	- 5000	- 10500
Bulinus truncatus	1220- 2440	25/42	0/7	0/4
Mercuria confusa (Frauenfeld) and *M. punica* (Letourneux and Bourguignat)	1220-10500	27/42	6/7	3/4
Hydrobia aponensis Martens	1220-10500	27/42	4/7	3/4
Melanoides tuberculata (Müller)	1220-10500	32/42	6/7	3/4
Melanopsis spp.	1550- 3580	19/42	4/7	0/4

Table 2. Occurrence of Tunisian gastropods according to total chloride concentration (ppm).

Species	Range	120 - 700	- 1500	- 3900
Bulinus truncatus	120- 602	25/45	0/6	0/2
Mercuria confusa and *M. punica*	120-3900	29/45	5/6	2/2
Hydrobia aponensis	120-3900	28/45	4/6	2/2
Melanoides tuberculata	120-3900	33/45	6/6	1/2
Melanopsis spp.	132- 956	19/45	3/6	0/2

a distinctly bitter or salty taste and *Bulinus* was not encountered, no analyses were done. Of the 53 stations where chemical data are known, only two were free of any mollusks. Within the ranges of analysis values, *Bulinus* was limited only in (1) high total electrolyte contents (expressed by electrical conductivity, Table 1) with an upper limit of 2440 μmho, and (2) high chloride concentrations (Table 2), the highest tolerated value being 602 ppm. As to these chemical characters all the commonly occurring prosobranch snails much exceeded the *Bulinus* threshold.

The calcium to magnesium ratios (Fig. 4) lying between 0.63/1 and 3.4/1 at the 53 stations obviously did not reach beyond the range tolerated by *Bulinus* in nature. Only the extremely high absolute concentrations of these cations (Ca >21 meq/*l* = 425 ppm; Mg >11 meq/*l* = 134.5 ppm) were avoided by *Bulinus*. The upper limit of carbonate hardness (total range 1.4 to 8.6 meq/*l*) where *Bulinus* lived was 4.7 meq/*l*.

Water from a sampling station near Gabès, Tunisia, where *Bulinus* did not occur, was brought to the laboratory and checked for its effect on Tunisian *Bulinus* snails. The water had an electrical conductivity (18°C) of 5200 μmho; calcium 23.5 meq/*l* = 471 ppm; magnesium 18 meq/*l* = 220.5 ppm; iron 0.03 ppm; carbonate content 4.8 meq/*l*; chloride 1118 ppm; nitrate - nitrogen 1.1 ppm; (the sulphate determination yielding 432 ppm was unreliable and should be neglected).

Snails were acclimatized to this water by passing them through three grades of dilution (original water/deionized water 50%, 75%, 85%) for 2 or 3 weeks each. In 100% water *Bulinus* snails survived for no more than one to two days (one snail eight days) and did not lay eggs. Simple dilution of original water with deionized water (Fig. 5) permitted egg laying, and the egg laying rate increased up to the ten-fold dilution where the medium contained one tenth of the values mentioned above.

In a last series of experiments the egg laying rate was examined in an artificial medium, where the Ca/Mg ratio was kept constant at 3.65/1 and the total electrolytes were raised by adding the chlorides of calcium and magnesium (Table 3). Egg laying rates were high in SFW 100 and remained at that level until the total electrolyte content was raised to more than 16.5 meq/*l* and a chloride concentration of 334 ppm. During the experiment (54 days) one snail died in group 3, and 4 snails died in group 4.

DISCUSSION

The very low egg laying rates in the Algerian snails may be considered strain-specific. This is mirrored by the low numbers of eggs per mass, which was about 2 to 3. For comparison, in the Tunisian strain the number of eggs/egg mass is about 11. In Fayoum, Egypt, it is about 8. These differences can be partially due to differences in snail size. Low reproductive rate in the Algerian strain, nevertheless, obviously does not hamper maintenance, as indicated by the successful rearing of these snails in tap water for 6 1/2 years,

Table 3. Egg laying rate of *Bulinus truncatus* in SFW 100 with addition of chloride but constant Ca/Mg ratio (3.65/1). 4 x 4 snails, 7 mm high, in each group. Egg numbers from 54 days of observation. Tunisian strain.

Group	Total Electrolytes Meq/l	Approximate Electrical Conductivity	Achieved by Adding Meq/l		Total Chloride ppm	Eggs/Snail/Day	Eggs/Egg Mass x ± s.d.
			$CaCl_2$	$MgCl_2$			
0 (Contr)	7.88	470	—	—	28	2.42	4.67 ± 0.55
1	11.51	900	2.85	0.78	157	2.38	5.05 ± 0.53
2	16.51	1500	6.77	1.86	334	2.54	5.10 ± 0.39
3	31.51	3300	18.55	5.08	866	1.12	2.96 ± 0.61
4	56.51	6300	38.17	10.46	1752	0.34	3.54 ± 0.37

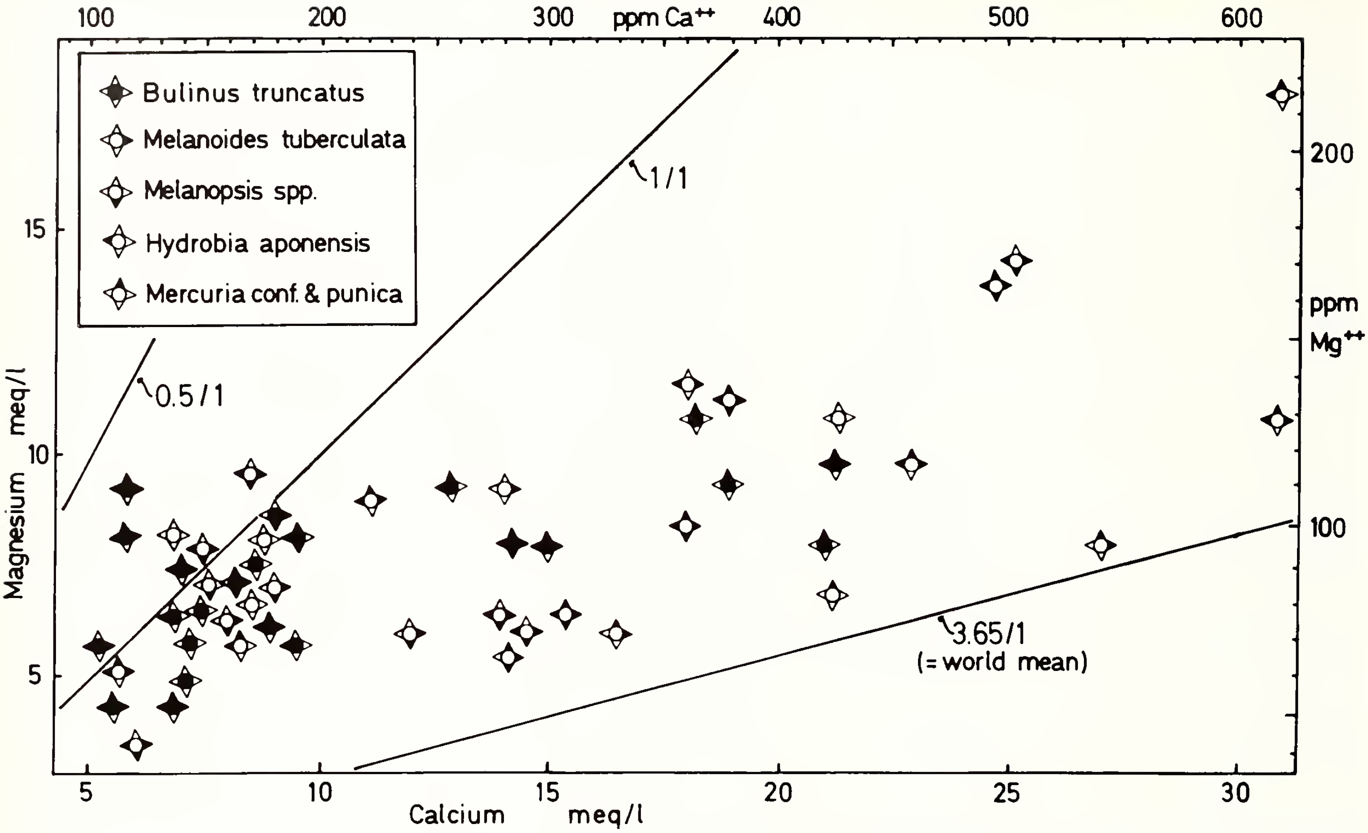

Fig. 4. Absolute calcium and magnesium concentrations and ratios in relation to gastropods collected at 53 sampling stations in southern Tunisia (1970 and 1971, both in spring).

where only three or four eggs per mass is normal.

Increased sulphate content more adversely affects egg laying than increased chloride content. Due to the lack of sulphate determinations in the field study, however, one cannot decide whether or not this anion limits distribution of *Bulinus truncatus* in Tunisia.

Adjusting the Ca/Mg ratios by adding magnesium as a chloride, though evidently better tolerated, primarily does not permit a decision as to whether the decrease in egg laying rates below the Ca/Mg ratio of 0.75/1 (Algeria) or 0.5/1 (Tunisia) was caused by an adverse Ca/Mg ratio or by the increased chloride concentration.

In regard to the Ca/Mg ratios, field distributions (Fig. 4) clearly demonstrate that all Tunisian water samples lie in a range between 3.4 and 0.63. This does not reach the experimentally determined value found to form the threshold for "normal" *Bulinus* reproduction (Fig. 2).

An effect of increased chloride concentration, using the same chloride amounts as in the Ca/Mg ratio variation, but a constant Ca/Mg ratio of 3.65/1, on the other hand, clearly shows that the significant drop of egg laying as well as eggs/mass lies between 334 and 866 ppm chloride. The highest field value in *Bulinus* habitats, 602 ppm, is in the same range. The upper limit in West Lybia, as found by Vermeil *et al.* (1952) (quoted by Deschiens, 1954), is in the same order

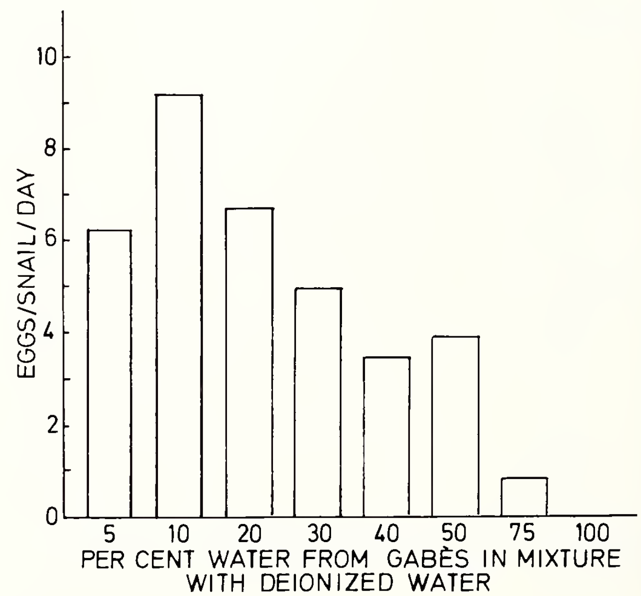

Fig. 5. Egg laying rates of *Bulinus truncatus* in original water from a *Bulinus* free irrigation canal north of the oasis of Gabès and in a series of dilutions. Mean values of counts over three weeks. Other conditions as in figure 2. Differences are significant (t-test: $p < 0.05$) between 75 and 50% and between 50 and 10%.

of magnitude, viz. between 530 and 980 ppm chloride, although Deschiens also quoted Marill (1953) who claimed to have encountered *Bulinus* in Algeria at a chloride content as high as 1530 ppm.

Chloride concentration of SFW was increased by adding $CaCl_2$ and $MgCl_2$ instead of NaCl, as usually done (Chu *et al.*, 1968; El Hassan, 1974, who used monoionic media that were completely nonphysiological), because high differences between total and carbonate hardness ("permanent" hardness) in Tunisia suggest that considerable amounts of calcium and magnesium occur in the form of chlorides and sulphates. Sodium, which was not determined due to the then inadequate analytical facilities, can therefore be present only in minor amounts. Similarly, the significant increase of the egg laying rate in the dilution series with water from Gabès (Fig. 5: 75% to 50%) was encountered when the chloride concentration dropped from 838 to 559 ppm. The natural upper limit of 602 ppm chloride lies between these two values. What ever significance may be attributed to the chloride for *Bulinus*, it must not be seen as isolated. The high absolute contents of total ions certainly play a role in limiting the distribution of *Bulinus* in Tunisia, and probably in other arid countries as well. This is indicated by the further increase in the egg laying rate after further dilution (Fig. 5), even far below the chloride threshold of around 600 ppm.

When electrolyte concentrations in Tunisia are compared with those in habitats of schistosome host snails of Africa south of the Sahara, the high levels in the arid zones are in a range that is certainly not tolerated by other species. In the Rhodesian "stream 1" of Harrison *et al.* (1966), where *Biomphalaria pfeifferi* was absent, not only was the calcium to magnesium ratio extremely low, viz. 0.05/1 (i.e. 5:62 ppm), but the water was also at the border between soft and medium, (*sensu* Williams, 1970). It contained no more than 5 ppm calcium whereas the "softest" water in Tunisia contained 97 ppm. Modifying standard freshwater (with 100 ppm Ca^{++}) to a ratio of 0.05/1 would have been possible. However, it would have meant a rise in the absolute electrolyte content to an unrealistic level. With the egg laying rates of *Biomphalaria pfeifferi* in their "stream 2" water, Harrison *et al.* (1966) demonstrated the role of absolute hardness. This water had a Ca/Mg ratio of 0.03/1 (i.e. 5.3:104.5 ppm). But while addition of $CaCl_2$ to stream 1 water up to 62 ppm (resulting in an equivalent ratio of 3.1/5.1 = 0.61) led to an increase from about 6 to 23 eggs per snail per fortnight, they did not succeed in raising egg laying in stream 2 water by adjusting the calcium content up to 104.5 ppm (corresponding to 5.2/8.6 = 0.61 equivalent ratio). In the original water the egg laying rate was nil, in the "adjusted" medium no more than 1.8 per fortnight. From this and other results (maximum respiration at 14 ppm calcium, Harrison, 1968; highest r_m-values at 12 ppm, Harrison *et al.*, 1970) they concluded that "medium" water (Williams, 1970; 5-40 ppm Ca^{++}) is optimal for *Biomphalaria pfeifferi*. A preference for soft to medium water may explain why *B. pfeifferi* do not live in arid climate zones as does, e.g. *Bulinus truncatus*.

Whether other species of *Bulinus* are adapted to hard or extremely hard water, as indicated by *B. truncatus*, must be examined. It is conspicuous, however, that some of the East African lakes, where transmission of only *Schistosoma mansoni* Sambon occurs, have low calcium concentrations, besides very low Ca/Mg ratios: Lake Albert (about 10 ppm Ca^{++}, Ca/Mg ratio about 0.18, Talling and Talling, 1965), Lake Edward (about 15 ppm Ca^{++}, Ca/Mg ratio about 0.16), Lake Victoria (about 10 ppm Ca^{++}, Ca/Mg ratio between 1.3 and 1.9). In the two former lakes *Bulinus* s.l. seem to be absent or at least rare (Dawood and Gismann, 1956), although these lake areas are not left vacant from shading in maps given by Brown (1980) for the *africanus* and the *truncatus* groups. Although generalizing ecological data (as suggested by the presence of several species of *Biomphalaria* in the Great Lakes, e.g. *B. stanleyi* (Smith), *B. smithii* (Preston), plus *B. sudanica* (Martens), and *B. choanomphala* (Martens), can lead to oversimplification, one may dare to say that *Biomphalaria* prefers rather soft to medium hard water, probably far below 100 ppm calcium, whereas *Bulinus* not only prefers hard water but also tolerates very hard waters, up to 425 ppm Ca^{++} (Fig. 4). Beyond these limits *Biomphalaria* and *Bulinus* spp. are probably no longer able to cope with osmoregulatory difficulties. The prosobranch snails (Fig. 4), which are regularly encountered in nearly all types of water bodies in southern Tunisia, evidently have no problems with the high chloride and total electrolyte concentrations (see Tables 1 and 2, Fig. 4). It can be speculated that the prosobranch freshwater snails do not show the physiological distance from their marine relatives that have been attained by freshwater pulmonates.

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